

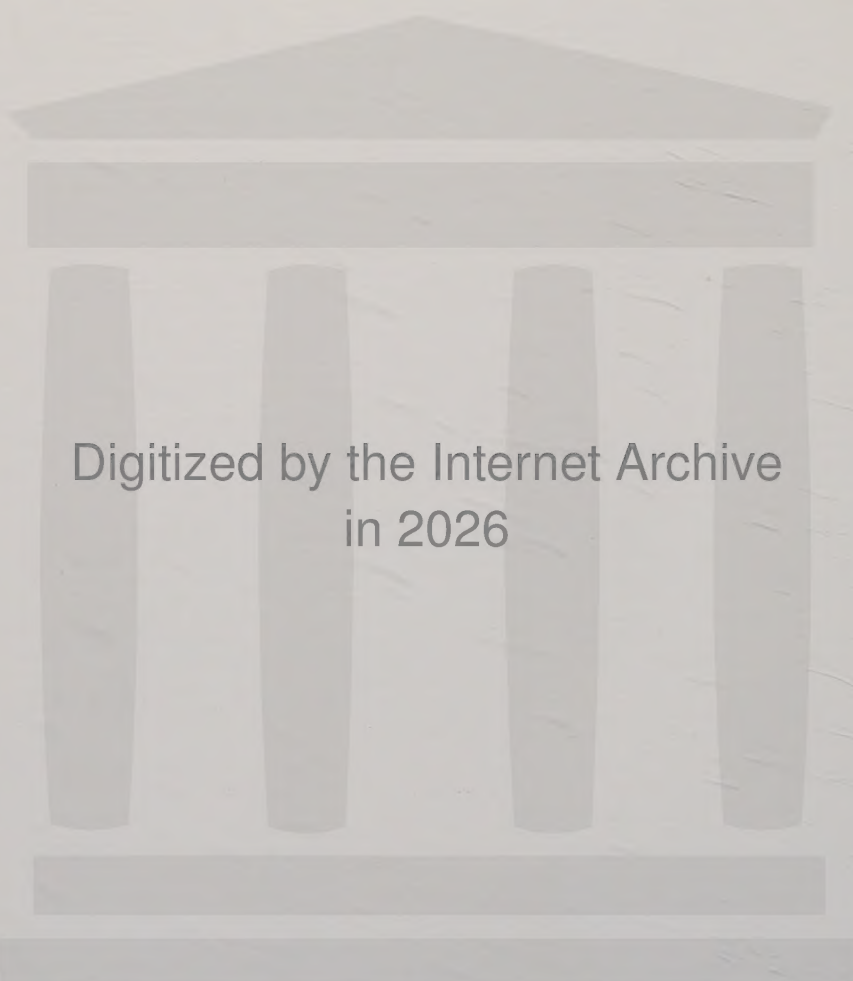
Studies on Antigen-induced Bronchial Anaphylaxis in Actively Sensitized Guinea-pigs.

**Differentiation of Two Models
with Respect to Immunological
Parameters and Drug Effects.**

by

Per Andersson

Lund 1982



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From Department of Zoofysiologi, University of Lund, Sweden

STUDIES ON ANTIGEN-INDUCED BRONCHIAL ANAPHYLAXIS IN ACTIVELY
SENSITIZED GUINEA-PIGS. DIFFERENTIATION OF TWO MODELS WITH
RESPECT TO IMMUNOLOGICAL PARAMETERS AND DRUG EFFECTS.

BY

PER ANDERSSON, F.K. VRML.

Akademisk avhandling som för avläggande av filosofie doktors-
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Title and subtitle Studies on antigen-induced bronchial anaphylaxis in sensitized guinea-pigs. Differentiation of two models with respect to immunological parameters and drug effects.			
Abstract: This thesis characterizes a model of reagin-mediated bronchial anaphylaxis in guinea-pigs designed to be used at research on anti-asthmatic drugs. Bronchial anaphylactic responses were estimated as changes in pulmonary resistance and dynamic lung compliance in anaesthetized guinea-pigs. 1 µg OA together with alum given i.p. was found to be an optimal dose for induction of a persistent state of responsiveness. Lower dose of OA did not induce responsiveness, whereas higher ones induced responsiveness which faded rather early. In animals primed with low doses of antigen, booster injections led to development of anaphylactic responsiveness. Cyclophosphamide (CY) given before a primary or a secondary antigen injection prolonged the ensuing anaphylactic response capacity. Analysis of serum homocytotropic antibody titre by PCA tests indicated that IgE-like antibodies mediated anaphylactic responsiveness after this kind of immunization and that pretreatment with CY increased IgE-biosynthesis. Immunization of guinea-pigs with high doses of OA without adjuvant induced anaphylactic response capacity which was found to be mediated by IgG₁-like antibodies. The use of mediator-antagonists indicated that both types of anaphylactic bronchoconstrictions were due to released histamine and SRS-A. Participation of prostaglandins and thromboxanes seems to be small. Various drugs affected the anaphylactic responses in the two systems in different ways. Aminophylline inhibited both the IgE- and IgG-mediated anaphylaxis in subbronchodilating doses. SCG and phospholipase A₂ inhibitors like mepacrine, p-bromphenacyl bromide and the glucocorticoids budesonide and hydrocortisone inhibited the IgE but not the IgG-mediated anaphylaxis.			
Key words Guinea-pigs, bronchial anaphylaxis, ovalbumin, IgE, IgG, PCA, cyclophosphamide, secondary response, sodium cromoglycate, aminophylline, glucocorticosteroids, phospholipase A₂ inhibitors, mediator-antagonists, acute-treatment, long-term treatment.			
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28/6 1982

Studies on Antigen-induced Bronchial Anaphylaxis in Actively Sensitized Guinea-pigs.

Differentiation of Two Models with Respect to Immunological Parameters and Drug Effects.

by

Per Andersson

Lund 1982

Studies on
Antigen-induced Bronchial
Asthma in Actively
Sensitized Guinea-pigs.

Differentiation of two models
with respect to immunological
parameters and drug effects.



By
F. J. Anderson, A. B. & C.

June 1983

PREFACE

This thesis is mainly based on studies reported in the following publications which will be referred to in the text by their Roman numerals:

I. P. Andersson.

Antigen-induced bronchial anaphylaxis in actively sensitized guinea-pigs. Pattern of response in relation to immunization regimen. *Allergy* 35, 65-71, 1980.

II. P. Andersson.

Antigen-induced bronchial anaphylaxis in actively sensitized guinea-pigs: Anti-anaphylactic effects of sodium cromoglycate and aminophylline. *Br. J. Pharmacol.* 69, 467-472, 1980.

III. P. Andersson.

Antigen-induced bronchial anaphylaxis in actively sensitized guinea-pigs. The effect of booster injection and cyclophosphamide treatment. *Int. Archs. Allergy Appl. Immunol.* 64, 249-258, 1981.

IV. P. Andersson & H. Bergstrand.

Antigen-induced bronchial anaphylaxis in actively sensitized guinea-pigs: Effect of long-term treatment with sodium cromoglycate and aminophylline. *Br. J. Pharmacol.* 74, 601-609, 1981.

V. P. Andersson & R. Brattsand.

Protective effects of the glucocorticoid, budesonide, on lung anaphylaxis in actively sensitized guinea-pigs: Inhibition of IgE- but not of IgG-mediated anaphylaxis. *Br. J. Pharmacol.* 76, 139-147, 1982.

VI. P. Andersson.

Effects of inhibitors of anaphylactic mediators in two models of bronchial anaphylaxis in anaesthetized guinea-pigs. (Accepted for publication in *Br. J. Pharmacol.*)

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INTRODUCTION

In general, bronchial asthma has been characterized by hyper-reactivity of the lower respiratory tract to various immunologic and non-immunologic stimuli. The mechanical obstruction of the small airways causing the asthma symptoms is due to constriction of bronchial smooth muscle, edema of the bronchial wall, and hypersecretion of mucus (Vaughan et al. 1973, Read & Townley 1978).

In the pathogenesis of human allergic asthma, hyperproduction of reaginic (IgE-like) antibodies and the reactions mediated by this antibody class seem to play a significant role (Ishizaka et al. 1966, Kay & Austen 1971, Orange et al. 1971, Johansson & Foucard 1978). Although there are reports of anaphylactic antibodies of the IgG-type in man (Bryant et al. 1973, 1975), the pathophysiologic importance of the IgG antibody-mediated reactions for the human allergic asthma symptoms is not clear (Ishizaka & Ishizaka 1973). In addition to the antigen-induced reactions an important component of allergen-induced asthma seems to be a non-specific hyperreactivity of the airway (Popa 1980, Hargreave et al. 1981). The cause of this non-specific hyperreactivity is not clear. At allergen exposure, an immediate asthmatic reaction develops within a few minutes. Such a reaction is often followed by a late asthmatic reaction developing after some hours (Dolovich & Hargreave 1981). In the literature, controversy exists whether late asthmatic reactions are mediated by IgE- or IgG₄-type of antibodies or both (Dolovich & Hargreave 1981, Gwynn et al. 1982).

The detailed mechanism of immediate type reagin-mediated allergic respiratory disease has repeatedly been reviewed (Quanjer 1977, Ishizaka & Ishizaka 1978b, Johansson & Foucard 1978, Hirata & Axelrod 1980, Ishizaka 1981). It has been proposed that

the anaphylactic reaction results from the interaction between the specific allergen and the cell-fixed IgE antibodies. Many competent cells may be involved in the reaction. In particular tissue mast cells within chopped lung fragments and basophil leucocytes have been extensively studied. However, due to difficulties in obtaining pure tissue mast cells, model studies at the cellular level have primarily been performed with rat serosal mast cells. It has been proposed that the cross linking of IgE receptors on these cells induces activation of phospholipid methyltransferases, and that the phospholipid methylation at the plasma membrane results in an increase in Ca^{2+} influx and a release of pharmacologically active compounds like histamine, slow reacting substance of anaphylaxis (SRS-A) and eosinophilic chemotactic factor. The late asthmatic reaction has been suggested to depend on a mast cell-derived factor, an inflammatory inducing factor of allergy (Oertel & Kaliner 1981). These compounds are thought to lead to the manifestations of asthma (Ishizaka 1981).

The role of the adrenergic and cholinergic nervous system in allergen-induced respiratory diseases also seems important. It has been proposed that the increased reactivity of the parasympathetic nervous system plays an important role in the production of asthma (Simonson et al. 1967, Boushey et al. 1981). On the other hand, stimulation of the β -adrenoceptors has been shown to relax bronchial smooth muscle and to decrease mediator release (Assem & Schild 1969, Reed 1974, Lefkowitz 1976, Sörenby 1977). Szentivanyi (1968) proposed that the primary defect in asthmatic subjects is a partial β -receptor blockade. Such a blockade might interfere with the homeostatic control of the smooth muscle and with mediator release.

The introduction of SCG (Altounyan 1967) created a great interest in developing new, more effective "anti-allergic" agents for prophylactic use in asthma. Most experimental animal models used in the search for such drugs are based on the "mediator

concept" of allergic disorders, because inhibition of mediator release was the supposed mechanism of action of SCG. One has then frequently used reagin-mediated immediate hypersensitivity reactions such as passive cutaneous anaphylactic reactions (PCA) in the rat and guinea-pig as a discriminating tool (Church 1978). Another main model has been to study the inhibition of histamine release from chopped guinea-pig or human lung. However, these approaches have generally led to disappointing clinical experiences. This fact stresses the importance of finding animal models with greater potential for clinical relevance.

Animal respiratory disease which resembles either reagin-mediated or non-reagin-mediated human disease may not occur, with the possible exception of certain rare cases in dogs. Patterson (1969) and Patterson & Kelly (1974) described dogs which had an IgE-mediated ragweed pollenosis, confirmed by cutaneous or respiratory challenge with ragweed pollen extracts. Both the clinical syndrom and the underlying immunology were similar to that of ragweed pollenosis in man. Patterson (1969), Patterson & Harris (1976) and Pare et al. (1976) described a model of allergic respiratory disease in rhesus monkeys which was very similar to the canine model of ascaris hypersensitivity. However, naturally occurring pollenosis has not been described in non-human primates (or any other species whatsoever). Against this background it was thought worthwhile to search for more useful animal models in lung anaphylactic studies.

Many years ago, Auer & Lewis (1910) attributed the fatal anaphylaxis in guinea-pigs to severe bronchoconstriction. The fact that the anaphylaxis has been considered to be mediated by IgG antibodies and that conflicting results have been obtained with SCG, has made the guinea-pig a less widely used animal in the testing of anti-allergic drugs (for ref. see Church 1978 and II). However during the last 10 years, an IgE-like antibody has been defined in the guinea-pig (for ref. see I). Cox (1978) has

extensively reviewed the techniques used to assess the degree of bronchoconstriction in guinea-pigs. However, no firmly demonstrated reagin-mediated asthma model in guinea-pigs exists where the effects of human anti-asthma drug has been unequivocally shown.

AIMS OF THE PRESENT INVESTIGATION

The aims of the present investigation are

1. To define suitable conditions for the induction in guinea-pigs of reagin-mediated bronchial anaphylactic reactivity in vivo and its precipitation at allergen challenge.
2. To characterize this model and to compare it with an IgG₁-mediated in vivo bronchoconstriction model.
3. To elucidate in general and specific ways the potential of the clinical relevance of such a model. For this purpose, the effects of different types of anti-asthmatic drugs have been examined in the model. The anti-asthmatic drugs chosen are SCG, AM and glucocorticoids.
4. To give aspects on the mechanisms behind the effect of these drugs.

METHODS

Animals

Outbred guinea-pigs of either sex (Dunkin-Hartley) bred by Sahlins, Malmö, Sweden. The age of the guinea-pigs at sensitization was 4-5 weeks and their weight was 250-300 g.

Drugs

Ovalbumin (OA), 1-isoprenaline hydrochloride, acetyl-beta-methylcholine, indomethacin, mepacrine (Quinacrine), adenosine, 2-Cl-adenosine, Sigma; Mebumal^R, aminophylline (AM), sodium-chloride (NaCl), ACO, Sweden; atropine sulphate, carbacholine chloride, histamine chloride, hydrocortisone acetate (micronized), Apoteksbolaget, Sweden; SCG, FPL 55712, Fisons UK; enprofylline, budesonide (micronized), AB Astra, Sweden; dimaprit hydrochloride, cimetidine (Tagamet^R), SK & F, UK; Evans Blue, Merck, BRD; cyclophosphamid (CY; Sendoxan^R), Pharmacia, Sweden; O-phthalaldehyd, BDH Chemicals Ltd, UK; mepyramine malate, May & Baker Ltd, UK; BW 755C, Wellcome Research Laboratories, UK; p-bromphenacylbromid, AB Draco, Sweden; Al(OH)₃, (F2200 Reheis Chemical Company) obtained by AB Astra, Sweden.

The compounds needed for the histamine determination and Krebs solution were analytically pure standard chemicals. The Krebs solution contained (mM): NaCl 118; KCl, 4.7; CaCl, 2.5; MgSO₄, 1.16; NaHCO₃, 25; KH₂PO₄, 1.18; D-glucose, 11.0.

Drug administration

OA was injected intravenously. Acute treatment with drugs was carried out by intravenous injections at different times before test (immediately before to 10 min. before).

Long-term treatment with SCG was performed by an i.p. injection once a day for 3 weeks, 5 days a week. The treatment was stopped 1 day, 3 days, or 7 days before test. In these series of experiments, the effect obtained with SCG after long-term treatment was compared with the effects seen after one i.p. injection given 1 day, 3 days, or 7 days before test.

Long-term treatment with AM was performed by oral administration twice a day following the same time schedule as SCG.

Systemic treatment with glucocorticoids was performed as one i.p. injection (50 mg/kg) 15-20 h before test or as two consecutive injections (50 mg/kg) 5 and 6 days before test. Local pretreatment with glucocorticosteroids was given by intratracheal instillation (0.5, 1.6 or 5 mg/kg) 2, 6, 20, or 48 hours before test. The glucocorticosteroids were suspended in a vehicle of carboxymethylcellulose-sodium 0.75 %, Tween 80 0.4 % and NaCl 0.7 % ad 100 %.

Sensitization procedures

Three major sensitization procedures were used:

A. The guinea-pigs were injected i.p. with OA 5 mg on day 0 and 10 mg on day 2. The OA was dissolved in saline, and the injection volume was 0.1 or 0.5 ml. Animals sensitized according to this sensitization procedure were shown to produce only IgG-like antibodies. In the following the anaphylaxis in guinea-pigs sensitized according to this procedure will be referred to as "IgG"-mediated.

B. The guinea-pigs were sensitized by one i.p. injection of 0.5 ml saline containing $\text{Al}(\text{OH})_3$ (1 mg or 100 mg). The OA doses were 0.1, 0.5, 1, 10, or 100 μg . The antigen was added to the adjuvant suspension 1 h before injection. Animals sensitized accord-

ing to this procedure were shown to produce both IgE- and IgG-like antibodies. In the following, the anaphylaxis in guinea-pigs sensitized according to this procedure will be referred to as "IgE"-mediated.

C. The guinea-pigs were injected with 30 mg/kg CY i.p. two days before primary sensitization with 1 μ g OA + Al(OH)₃, 100 mg or 10 μ g OA + Al(OH)₃, 100 mg or two days before the booster injection of 1 μ g OA + Al(OH)₃, 100 mg. CY was dissolved in distilled water.

Respiratory measurements

The animals were anaesthetized with pentobarbital (Mebumal^R, 70 mg/kg) i.p., tracheotomized and ventilated by a Braun constant volume respirator (frequency 60-70/min, volume 5-7 ml/kg). Pulmonary mechanics, i.e. lung resistance (R_L) and dynamic lung compliance (C_{DYN}), were estimated by the Amdur & Mead (1958) method modified for anaesthetized guinea-pigs, as given below. Airflow ($\dot{V}$) was measured by a mesh screen pneumatograph (Fleisch no. 000), connected to a Statham differential pressure transducer (PM 15). Tidal volume (V_T) was determined as the electrical integral of airflow. The transpulmonary pressure (P_{TP}) was determined by connecting one inlet of a differential pressure transducer (Statham PM5) to a needle inserted through the 5th or 6th intercostal space to measure the intrapleural pressure, and the other inlet to a small rubber tubing placed between the endotracheal cannula and the pneumotachograph to measure the intratracheal pressure. Output signals representing P_{TP} , $\dot{V}$ and V_T were registered simultaneously on a Grass polygraph model 7. R_L and C_{DYN} were determined by manual calculations according to the Amdur & Mead (1958) method. The animals were challenged at various (9 to 70) days after sensitization with various doses of OA (2 to 1000 μ g/kg) injected intravenously through the left jugular vein. Throughout the entire experiments, blood pressure

was registered by a catheter inserted in the right carotide artery. The heart frequency was determined by a tachograph (Grass model 7P4) triggered by the blood pressure.

Isolated guinea-pig trachea

The animals were stunned with a blow on the head. The tracheas were removed, spirally cut and placed in organ baths filled with Krebs solution (37° C), aerated with 95 % O₂ and 5 % CO₂. The tracheas were connected to force displacement transducers (FT03). The registrations were made on a Grass polygraph model 7. The initial tension was 1.5 g. The preparations were contracted by adding a single dose of carbacholine (0.2 - 0.3 µg/ml) or by running cumulative dose response curves with carbacholine or histamine. Relaxation was obtained by adding isoprenaline in increasing concentrations. The effect of each increase in dose of the compounds was calculated as per cent of the maximum effect of the compound. In the experiments where effects of glucocorticosteroid on isoprenaline sensitivity were examined in vitro the steroids were dissolved in ethanol (final bath concentration = 0.25 %). The other compounds used were dissolved in saline.

Histamine release from chopped lung tissue

The animals were stunned with a blow on the head. The lungs were removed and perfused through the pulmonary artery with chilled Krebs solution. They were dissected free from major airways and blood vessels, and chopped with scissors. The fragments were washed 4 times in additional Krebs solution and weighed into 100 mg portions each containing 5-6 fragments. Each histamine release assay was performed in a total volume of 1 ml Krebs solution. To start the release reaction, OA (final concentration 0.01, 1.0, 10.0, or 100.0 µg/ml) was added, the incubation time

being 30 minutes. The reaction was terminated by placing the tubes on ice, supernatants were removed and fresh medium was added. The remaining histamine content of the lung fragments was extracted by boiling the fragments for 8 minutes after the release process. The tissue was removed from the samples and supernatants were acidified with 70 % perchloric acid and precipitation of protein was carried out for 15 minutes. The samples were centrifuged at 800 g for 10 minutes and the histamine content of the supernatant fraction was quantified spectrophotofluorometrically essentially as described by May et al. (1970). Separate experiments showed that extraction of histamine from the supernatants did not significantly change the results; this step was therefore omitted in the present investigation. The per cent histamine release was calculated according to the following formula:

$$\frac{\text{antigen-induced release} - \text{spontaneous release}}{\text{total tissue content} - \text{spontaneous release}} \times 100$$

Determination of homocytotropic antibody titre

PCA reactions were used according to the principles given by Watanabe & Ovary (1977) to estimate serum homocytotropic antibody titre. Blood samples were obtained either by cutting the carotid artery in stunned animals or by sampling (3.0 ml) from a catheter inserted in the carotid artery before the provocation of animals undergoing respiratory measurements. In each experiment, a pool of sera (0.5 ml of each) was prepared and stored at -80°C and thawed just before use. Each serum was examined by passive cutaneous anaphylaxis both before and after heat treatment (56° C; 1 or 4 h). 0.1 ml of the serum examined, appropriately diluted in saline, was injected into the shaved abdominal skin of normal guinea-pigs. After a latent period of 4 h, 4 days, or 7 - 14 days, the animals were challenged by an i.v. injection of 2 mg OA together with 10 mg/kg Evans Blue.

These latent periods are considered to reflect mainly the activities of IgG_{1a}-, IgG_{1b}- and IgE-like antibodies, respectively. The antibody titre was estimated by the end point dilution technique. Titres given are the highest dilution that showed bluening (minimum spot diameter 5 mm) in four out of six animals.

Statistics

Statistical evaluation by Student's t-test. The dose-response curves were compared by Parallel Line Assay (Finney 1950).

RESULTS AND DISCUSSION

The present series of experiments was initiated with the aim of developing a guinea-pig test model to human allergic asthma to be used in therapeutic oriented research. It is desirable that such a model should meet the following four criteria: (1) suitable techniques for quantification of lung function changes should be applicable, (2) a state of persistent bronchial allergy of the reagin-mediated type should be induced, (3) a bronchial non-specific hyperreactivity should be present, and (4) an appropriate pattern of response to clinically effective drugs should be recorded.

A. Characteristics of the respiratory measurements employed

In asthma both central and peripheral airways may be obstructed (Pattersson & Kelly 1974, Bouhuys 1976, Reed & Townley 1978, Pride 1978, McFadden 1978). We have chosen to follow the bronchial response according to the general principles described by Hahn & Nadel (1979). Specifically the method used by Amdur & Mead (1958) in unanaesthetized guinea-pigs was followed (I). According to those principles, R_L is thought to assess changes in airway caliber primarily in large bronchs and C_{DYN} primarily in the peripheral part of the lung. However, the use of anaesthetized, artificially ventilated animals also permits a rough evaluation of the site of action of a drug. Thus, in the cat it has been shown that histamine given intravenously causes a relatively weak change in R_L compared with in C_{DYN} (Colebach et al. 1966, Andersson & Persson 1977). This agrees with the findings in vitro where histamine has been shown to contract airways of approximately 1 mm in diameter while no effect was seen on large bronchs (Persson & Ekman 1976). Changes in R_L and C_{DYN} caused by antigen challenge of sensitized guinea-pigs have

previously been considered by several authors to mimic changes seen in asthma (for ref. see Drazen 1977, Broder et al. 1978). In the present experiments an almost instantaneous change in lung mechanics was observed after intravenous challenge of appropriately sensitized animals with a sufficient dose of allergen. There is no clear-cut indication in the present data that the response in sensitized animals to an antigen challenge differs qualitatively with respect to changes in R_L or C_{DYN} , indicating involvement of both small and large airways in the response. Furthermore, there is no indication that the nature of the antigen-induced response should vary qualitatively with immunization regimen.

B. Characterization of anaphylactic response capacity and nature of antibodies mediating the anaphylactic response.

In the pathogenesis of human allergic asthma, IgE antibody-mediated reactions seem to play a major role (for ref. see Johansson & Foucard 1978). However, there are some investigations which show that type I allergic reactions may also be mediated by IgG antibodies. A heat stable IgG antibody capable of mediating immediate skin test reactions has been described in patients with food allergies by Parish (1970) and in asthmatic subjects by Bryant et al. (1973). Thus, according to present knowledge, an animal model of human allergic bronchial asthma should preferably include a state of bronchial reagin-mediated sensitivity to the antigen used. The animals should show a secondary IgE response when re-exposed to the antigen. The anaphylactic bronchoconstriction in the guinea-pig has been used as an asthma model, but lately it has been considered less suitable because of the conflicting results obtained with SCG and because IgG₁, but not IgE, has been considered to be the major homocytotropic immunoglobulin class of this species. In recent years, it has been shown that suitably sensitized guinea-pigs are able to produce IgE antibodies (for references, see (I) and Cox 1978). Hicks & Okpaka (1968) examined in some detail the

influence of a single dose of antigen in the development and the duration of anaphylactic reactivity. However, a model of reagent-mediated bronchoconstriction in guinea-pigs fulfilling the four criteria expressed above has not yet been documented.

As low doses of antigen injected i.p. together with alum cause a sustained production of IgE antibodies in mice and rats (Jarrett 1978, Tada 1975) and as the i.p. route of immunization in mice has been found to produce the same pattern of IgE response to antigen as the intratracheal instillation (Gerbrandy & Bienenstock 1976), we have used i.p. injection of OA together with alum in the guinea-pig in an effort to produce IgE-mediated bronchial anaphylactic reactivity. We found that the most pronounced and sustained bronchial reactivity to low OA challenge doses was achieved when 1 μ g of OA was used. A lower dose (0.1 μ g of OA) failed to induce response capacity. Higher doses of OA (10 or 100 μ g) produce a comparably shorter persistence of the bronchial reactivity (I).

There is as yet no way of assessing IgE antibodies in guinea-pigs other than with PCA reactions. It has been shown in the rat system that the correlation between homocytotropic antibody level estimated by PCA and by radioimmune assay techniques is good (Pauwels et al 1977). However, the correlation is not unequivocal between the serum IgE antibody titre on the one hand and the bronchial sensitivity, the skin reactivity, or the capacity to release mediators from lung tissue on the other (Church 1975, Jarrett & Stewart 1973, Yamamoto et al. 1974, Stotland & Share 1974). One possible reason for this might be the existence of tissue specific antibodies. However, as will be discussed later, the present investigation shows that a booster dose or cyclophosphamide treatment induces a high titre of serum antibodies, which in PCA tests behave like IgE antibodies according to the criteria of Ovary et al. (1976) and Watanabe & Ovary (1977). This high level of IgE antibodies is correlated to a high degree of bronchial reactivity. Therefore, PCA is con-

sidered a relevant technique to estimate the lung sensitivity reagenic antibodies. We found moderate titres of heat-sensitive antibodies mediating PCA reactions after a 14 day latency period in pools of sera from guinea-pigs immunized for 14 days with 1 μ g or 10 μ g OA in alum (I). Thus, these groups of guinea-pigs synthesize IgE antibodies early in the development of the sensitized state. Unpublished results also show that i.v. administration of large volumes (5 ml) of sera from these guinea-pigs transfers a weak persistent bronchial allergic reactivity. This was abolished by heat treatment of the sera.

Based on the findings in paper (I) guinea-pigs sensitized with 1 μ g OA in alum has become the most important system for testing anti-allergic drugs in the present investigation. It must be noted that the titre of both IgG₁ and IgE antibodies in sera from these animals had decreased on day 50 compared with those observed 14 days after immunization (I). Still there is a high degree of anaphylactic reactivity even 70 days after sensitization. However, IgE antibodies have been shown to cause a persistent increased allergic response capacity (for ref. see Ishizaka & Ishizaka 1978). Studies on the kinetics of binding between IgE antibodies and receptors on target cells like the basophils or the rat mast cells have shown that the IgE antibody has a high affinity and a low dissociation constant which could explain why the sensitization is so persistent (for ref. see Ishizaka & Ishizaka 1978b). Furthermore, Jarrett & Stewart (1973) suggest that detectable levels of IgE antibodies do not appear in serum until IgE-fixing sites in pertinent tissues have been saturated. Thus, an explanation of the results found in paper (I) could be that there is an initial production of IgE antibodies which fix to lung mast cells and stay attached for several weeks. Alternatively, there could be a continuous production of low amounts of IgE antibodies which does not saturate the receptor sites on lung mast cells. With this background, we interpret our data as showing that the major part of the bronchial anaphylactic reactivity in guinea-pigs immun-

ized with low doses of OA in alum, is mediated by IgE-like antibodies. This interpretation is strengthened by the observations discussed below on the effect of cyclophosphamide treatment of animals before immunization.

The non-IgE guinea-pig homocytotropic antibody termed 1 is preferentially produced by intraperitoneal injection of high doses (100-1000 μ g) of antigen (Benacerraf et al 1963). In the present investigation we injected guinea-pigs intraperitoneally with high doses of OA (5 mg (day 0) + 10 mg (day 2)) in saline to serve as controls presumably possessing only IgG antibody-mediated anaphylactic reactivity (Bloch et al. 1963). We found that these animals developed a low degree of bronchial reactivity (I), i.e. they reacted only to higher doses of antigen, but then developed responses indistinguishable from those seen in animals given low doses of OA in alum. Examination of the serum antibodies with PCA showed only IgG antibodies (I).

C. Effect of cyclophosphamide (CY) and/or a second antigen-injection

In recent years, the role of the type of antigen, the antigen dose, and the role of adjuvant for the primary induction of reaginic antibody production and for the ability to obtain secondary reagin response have been extensively investigated in rodents. A growing body of evidence indicates that the synthesis of IgE antibodies is controlled separately from that of other immunoglobulins and much attention has been paid to the regulating functions of T cells, especially the role of suppressor T cells (Tada 1975, 1978, Ishizaka & Ishizaka 1978b, Jarrett 1978, Katz 1977, 1978 a,b). It has been observed that the IgE antibody response is more susceptible to antigen-specific suppressor T cells than the IgG antibody response is (Kishimoto et al. 1976, Suemura et al. 1977). X-irradiation or CY treatment before the antigen administration to low IgE responder phenotypes converts these animals into high ones with little or no effect on the IgG

antibody production (Chiorazzi et al. 1976, 1977, Watanabe et al. 1976, Katz 1978 a,b, Graziano et al. 1981). Various types of experiments show that the enhanced IgE antibody response after this kind of treatment is probably due to inhibition of antigen-specific and/or antigen-non-specific suppressor T cells (Chiorazzi et al. 1976, 1977). Although the above mentioned experiments show a selectively enhanced IgE production, there have been reports of increased IgG responses after CY treatment (Debr e et al. 1976, Gagnon & MacLennan 1979).

The results in the present investigation show that guinea-pigs primed with only 0.5 μ g OA and a low dose of alum show no bronchial reactivity at challenge with OA. However, after a booster dose of antigen these animals show a clear-cut anaphylactic reactivity which is reflected by a rise of both IgG- and IgE-like antibody titres (III).

Guinea-pigs primed with 10 μ g OA together with alum develop a transient bronchial reactivity, the fading of which is reflected in the antibody titres. Examination with PCA of the antibody titres after a booster dose injection to guinea-pigs sensitized by 10 μ g OA together with alum shows an increased production of IgG-like antibodies but only a slight increase in the IgE-like antibody titre. However, despite the high titre of IgG antibodies seen after the booster injection, no increased bronchial reactivity was seen (III). CY treatment before the primary sensitization or the booster administration results in an increased bronchial anaphylactic response when the animals are challenged with OA. The increased anaphylactic reactivity is reflected in increased IgE antibody production. However, CY treatment results in no increase or only a slight increase in the IgG-like antibody production in addition to that already accomplished by the primary sensitization or the booster dose administration. It has been argued that the IgE antibody production is subject to feedback control by IgG antibodies directed against the same antigen (Tada 1975). This might explain

the effect described above. However, despite the high IgG-like antibody titre seen after the booster injection, a marked increase in the IgE-like antibody titre was seen when CY treatment preceded the booster. This would indicate that no IgE antibody synthesis blocking activity was exerted by the IgG antibodies produced. Furthermore, these data indicate that IgG antibodies exert no blocking activity on the antigen-induced bronchial anaphylaxis. We suggest that the 10 μ g dose of OA used for primary sensitization activates non-specific (or precursors of antigen-specific) CY-sensitive suppressor T cells, which are responsible for the early termination of the primary response and/or the inhibition of the booster response.

D. Relative importance of different mediators in inducing acute bronchoconstriction/relaxation

IgE antibodies attach themselves to the surface of mast cells and blood basophil leucocytes, and the subsequent interaction between the antigen and antibody leads to the release of mediators participating in the anaphylactic reaction. During the last few years, the understanding of the mechanism behind the release of anaphylactic mediators mediated by IgE antibodies has increased. With rat mast cells or human basophils it has been shown that bridging of IgE receptors induces activation of methyltransferases which induces a series of phospholipid methylations. Simultaneously with this, production of cyclic AMP occurs (for ref. see Ishizaka 1981). The stimulation of phospholipid methylation at the plasma membrane results in an increase in Ca^{2+} influx concomitant with histamine release. In this process the Ca^{2+} -dependent phospholipase A_2 seems to play an important role. Treatment of rat basophil leukemia cells (McGivney et al. 1981) or human basophils (Marone et al. 1981) with mepacrine or p-bromphenacylbromide, compounds which block phospholipase A_2 in various systems (Volwerk et al. 1974, Vargaftig 1977), results in a decreased antigen- or anti-IgE-induced histamine release.

1. Arachidonic acid metabolites

Activation of purified rat mast cells and rat basophil leukemia cells by antigen or anti-IgE also leads to the release of arachidonic acid from membrane phospholipids, by action of phospholipase A₂ (for ref. see Hirata & Axelrod 1980). Arachidonic acid is transformed through the cyclooxygenase pathway to prostacyclins, prostaglandins and thromboxanes or by the lipoxygenase pathway leading to production of SRS-A (for ref. see Lewis & Austen 1981). Recent research has led to the identification of the principal active constituents of SRS-A, leukotriens C₄ and D₄ (Bach et al. 1980, Lewis et al. 1980).

SRS-A has long been suspected of playing a part in the type I allergic reaction (for ref. see Piper 1977, Borgeat 1981, Lewis & Austen 1981). Observations in animal studies indicate an effect on airway smooth muscle contractility both in vivo and in vitro. During the last few years, the compound FPL 55712, an antagonist of SRS-A, has been used in several immunopharmacological studies to define the role of SRS-A in anaphylactic reactions (Augstein et al. 1973, Chand 1979). The fact that FPL 55712 has a very short biological half-life and has to be given almost immediately before challenge, poses great difficulties in its use in human and animal studies. However, FPL 55712 given as an aerosol has been shown to exert inhibitory effect on a bronchoconstriction induced by nebulized LTC and LTD in man (Holroyde et al. 1981). Furthermore, high doses of FPL 55712 given intravenously immediately before antigen challenge have been shown to block SRS-A-induced bronchospasm in guinea-pigs (Sheard et al. 1977).

We show in the present investigation that phospholipase A₂ inhibitors like mepacrine or p-bromphenacylbromide inhibit the "IgE"- but not the "IgG"-mediated bronchial constriction. The present investigation also shows that FPL 55712 to some extent

inhibits the antigen-induced bronchospasm in guinea-pigs sensitized to produce both IgE and IgG antibodies. Furthermore, the participation of lipoxygenase derived products in the "IgE"-mediated bronchospasm was strengthened by the use of BW 755C, a compound mainly acting as a lipoxygenase inhibitor (Higgs et al. 1979) (VI). These results indicate that SRS-A at least partially contributes to the anaphylactic lung reaction in guinea-pigs.

The role of the products derived by the cyclooxygenase pathway in the anaphylactic lung reaction is controversial. There are reports of antigen-induced production of prostaglandins and thromboxanes in various species including man and guinea-pig (for ref. see Piper 1977, Al Ubaidi & Bakhle 1980, Spannhake et al. 1981). However, it has been observed that the cyclooxygenase inhibitor indomethacin has no effect on the antigen-induced bronchoconstriction in the guinea-pig (Michoud et al. 1976), and asthmatic patients showed no improvement in asthmatic symptoms nor did they become resistant to antigen challenge after indomethacin pretreatment (Smith 1975).

Indomethacin has a strong blocking action on the bronchospasm induced by arachidonic acid infusion, suggesting that such a spasm is induced by thromboxane A₂ or contracting prostaglandins (VI). However, indomethacin increased both the antigen-induced "IgE"-and "IgG"-mediated bronchoconstriction (VI). These results indicate that the antigen-induced bronchospasm in guinea-pigs is not due to cyclooxygenase-derived products. Rather, they conform to reports indicating that indomethacin in doses that inhibit the formation of cyclooxygenase products, augment the release of chemical mediators from sensitized guinea-pig lung (Engineer et al. 1978, Blackwell & Flower 1978, Piper et al. 1979, Hitchcock 1980). Similar results have been obtained in human lung (Walker 1973) after indomethacin treatment and after treatment of human basophils with indomethacin or non-steroidal anti-inflammatory compounds (Marone et al. 1979, Wojnar et al. 1980). Although it was originally suggested that these effects were due to sup-

pression of cyclooxygenase products like prostacyclin or PGE_2 , which have been shown to inhibit mediator release or to relax tracheal smooth muscle contracted by histamine (Lichtenstein & Henney 1972, Tauber et al. 1973, Orehek et al. 1973, Engineer et al. 1978) more recent evidence indicates that these effects are related to a re-direction of arachidonic acid metabolism to the lipooxygenase pathway (Hamberg 1976, Morris et al. 1980). This may result in an increased production of 5-HPETE which has been shown to enhance histamine release from human basophils (Peters et al. 1981) and/or to increase the production of SRS-A (Piper et al. 1979). We also showed that antigen administration to guinea-pigs pretreated with mepyramine and indomethacin results in a marked bronchospasm which is dose-dependently inhibited by FPL 55712 and mepacrine (VI). These results indicate production of SRS-A. As high doses of mepyramine were used during this experiment one cannot evaluate whether an increased production of histamine occurs.

In conclusion, SRS-A plays a role both in the "IgE"- and the "IgG"-mediated anaphylactic bronchoconstriction.

2. Histamine

Histamine is stored predominantly in tissue mast cells and circulating basophils and is released in response to antigen-antibody interactions. It has been shown to interact with two types of specific cell receptors, H_1 and H_2 (Black et al. 1972) in several organs. In the lung, histamine causes contraction of the smooth muscle by interaction with H_1 -receptors (for ref. see Plaute 1979). Another event mediated by histamine relevant to the symptoms in asthma is the proposed ability to cause bronchoconstriction via the vagal nerv (for ref. see Boushey et al. 1980). Histamine also increases the vascular permeability in blood vessels. This action is located at postcapillary venules where histamine produces partial disconnection of endothelial cells. This effect of histamine would contribute to the forma-

tion of edema during anaphylaxis (Persson et al 1982). By stimulation of H_2 -receptors on human basophils, histamine has been shown in vitro to inhibit mediator release (Bourne et al. 1971). Thus, histamine may exert a feedback inhibition of its own release. However, Kaliner (1978) found no effect of H_2 -antagonist on mediator release in human lung questioning the importance of this mechanism in that tissue. The negative practical experience with H_1 -blockers (for ref. see Karlin 1972) and histamine release inhibitors (Church & Gradidge 1980) makes the role of histamine as a main mediator of asthma questionable.

In the present investigation, mepyramine, an H_1 -antagonist, caused a dose-dependent decrease of the antigen-induced "IgE"-and "IgG"-mediated anaphylaxis indicating that histamine plays an important role in both these types of anaphylactic reactions. However, it was found that at equal OA concentrations more histamine was released in lungs taken from guinea-pigs sensitized to produce IgE antibodies than from those sensitized to produce only IgG antibodies (IV,V). Administration of the H_2 -agonist dimaprit caused a slight but not significant inhibition of the anaphylactic reactivity. When the H_2 -antagonist cimetidine was tested, no effect was seen on the antigen-induced bronchoconstriction (fig. 1). These results indicate that histamine plays a more dominant role for the development of the anaphylactic bronchoconstriction in the guinea-pig than in man.

3. Acetylcholine

Histamine has been shown to trigger a bronchoconstriction through an "irritant" receptor, parasympathetic reflex mechanism (Widdicombe 1963, Boushey et al. 1980). The mediator of this reaction is acetylcholine (Ach), the parasympathetic neurotransmitter. Atropine is the classic muscarinic anticholinergic agent, blocking the effect of Ach at postsynaptic cholinergic receptors. The question whether or not atropine is effective against

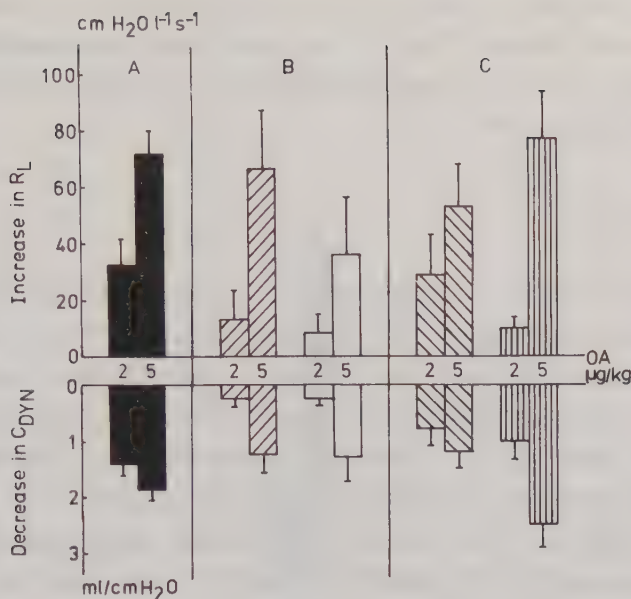


Figure 1. Antigen-induced increase in lung resistance (R_L) and decrease in compliance (C_{DYN}) (mean values, vertical lines show s.e.mean) after acute treatment with dimaprit and cimetidine in anaesthetized guinea-pigs sensitized with 1 μ g OA + Al(OH)₃, 100 mg. The animals were challenged i.v. with 2 and 5 μ g/kg of OA day 42 after sensitization. ■ Control response. ▨ Dimaprit 1 mg/kg given i.v. 2 min. before test. □ Dimaprit 10 mg/kg given i.v. 2 min. before test. ▩ Cimetidine, 10 mg/kg given i.v. 5 min. before test. ▤ Cimetidine 30 mg/kg given i.v. 10 min. before test. n=5-8.

an antigen-induced bronchial anaphylaxis in experimental animals and in asthmatic patients is not yet settled (for ref. see Gold 1978). Drazen et al. (1975) showed that atropine modified an anaphylactic bronchoconstriction in unanaesthetized guinea-pigs.

Anaesthesia has been shown to depress reflexes (Jackson & Richards 1977a). However, Mills & Widdicombe (1970) showed that to some degree atropine depressed the antigen-induced bronchoconstriction even in anaesthetized guinea-pigs. The present results show that acute administration of atropine has a significant effect on the OA-induced changes in R_L and C_{DYN} in

guinea-pigs sensitized to produce IgE-like antibodies, whereas no effect was seen in guinea-pigs sensitized to produce only IgG-like antibodies. Still, as shown above both responses are due mainly to histamine and SRS-A. We have no clear-cut explanation to these differences.

4. Adrenalin

Unpublished results of the present investigation show that propranolol increases the antigen-induced, "IgE"-mediated anaphylactic bronchoconstriction in anaesthetized guinea-pigs (fig. 2). Similar results have been reported by Collier & James (1967) and Piper et al. (1967). These observations indicate that liberated catecholamines take part in the regulation of guinea-pig anaphylaxis. The liberation of catecholamines could be due to a direct action of histamine on the adrenal medulla or to a reflex release caused by hypoxia.

The results obtained in the model for "IgE"-mediated bronchoconstriction fit into the theories reviewed by Hirata & Axelrod (1980) and Ishizaka (1981). The mechanism behind the anaphylactic response mediated by antibodies of the IgG-type has been very little investigated. Ishizaka & Ishizaka (1973) did not consider antibodies of the IgG-class important in inducing mediator release during the immediate type allergic reaction, mainly because of its low affinity for receptor sites on mast cells and basophils. However, in the in vivo situation circulating IgG antibodies may form immune complexes that fix to platelets (Movat et al. 1965, Mueller-Eckhardt & Lusher 1968, Henson & Spiegelberg 1973) or polymorphonuclear leukocytes (Weissman et al. 1971, Petersson 1975, Cammusi et al. 1977) thereby inducing release of pharmacologically active substances. Furthermore, immunocomplexes have been shown to activate the complement system (for ref. see Müller-Eberhard 1975). Mielens

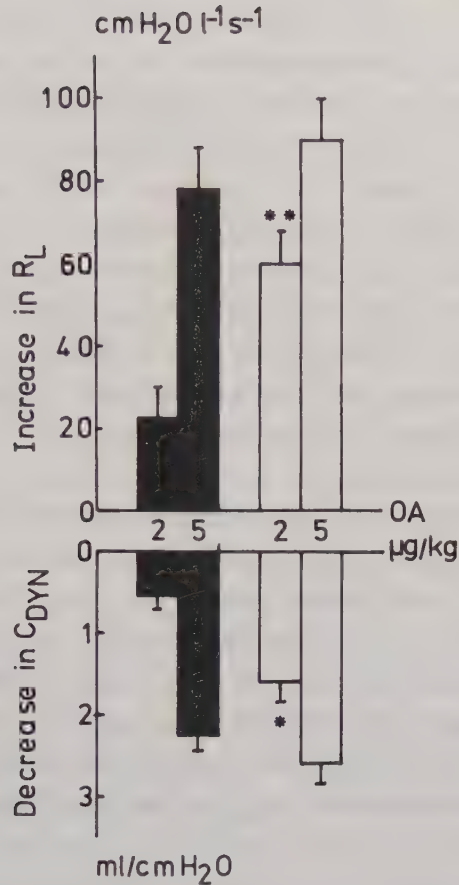


Figure 2. Antigen-induced increase in lung resistance (R_L) and decrease in compliance (C_{DYN}) (mean values, vertical lines show s.e.mean) after acute treatment with propranolol in anaesthetized guinea-pigs sensitized with 1 μ g OA + $Al(OH)_3$, 100 mg. The animals were challenged i.v. with 2 and 5 μ g/kg of OA day 42 after sensitization. ■ Control response. □ Propranolol 1 mg/kg given i.v. 10 min. before test. n=6. * 0.05 < P < 0.01 ** 0.01 > P > 0.001.

et al. (1981) showed that a bronchoconstriction induced by intravenously injected immune complexes in anaesthetized guinea-pigs seems to depend on the activation of complement and products derived by the cyclooxygenase pathway of the arachidonic

acid metabolism, but mepyramine was without inhibitory effect. It has been shown that anaphylatoxins can induce release of histamine from guinea-pig mast cells in vitro (Mota 1959, Schmutzler et al. 1977). However, Regal & Pickering (1981) have shown that a C_{5a} -induced contraction in the guinea-pig trachea was mediated by a non-histaminic compound, which was suggested to be SRS-A. On the other hand, the present investigation shows that histamine is released at antigen challenge in vitro from lungs taken from guinea-pigs sensitized to produce only IgG antibodies (IV, V). Furthermore, mepyramine has a marked inhibitory effect and indomethacin shows no inhibitory effect on an antigen-induced "IgG"-mediated bronchoconstriction in vivo (V, VI). These results indicate that the role of immunocomplexes and complement activation can be questioned.

There are reports of IgG-mediated release of anaphylactic mediators like histamine and SRS-A from rat mast cells (Moodley & Mongar 1981) and guinea-pig lung in vitro (Forsberg & Sörenby 1981, VI). Thus, the release described by us of histamine and SRS-A induced by IgG antibodies, could be derived from mast cells. There are indications of separate receptors for IgG and IgE antibodies on mice mast cells (Daeron et al. 1980). This observation together with our findings of different effects with proposed phospholipase A_2 inhibitors, indicate the involvement of different activation mechanisms in the "IgE"-and "IgG"-mediated anaphylactic reaction.

E. Effects of anti-allergic drugs

1. Sodiumcromoglycate (SCG)

SCG does not possess direct anti-inflammatory or bronchodilatory properties, but when inhaled prior to antigen exposure it inhibits at least partially, the development of immediate and late allergic bronchial reactions in asthmatic subjects. Because

of this effect profile, SCG is of particular interest as an anti-asthma drug whose mode of action is purely prophylactic. However, the exact mechanism of action is not known.

The results obtained in early studies suggested that SCG was active only as a specific inhibitor of IgE-mediated reactions (Altounyan 1967, Bryant et al. 1973, 1975). The drug's mode of action was thought to be inhibition of IgE-mediated mast cell mediator release. Other studies showed an effect of SCG also in extrinsic asthma due to its ability to stabilize mast cells independently of the stimulus applied and thus prevent the release of mediators (for ref. see Brogden et al. 1974, Anderson et al. 1975). Recently, results have been presented that question the relative importance of inhibition of mediator release as the mechanism of action of SCG in the treatment of asthma (Church & Gradidge 1980). Furthermore, SCG inhibits bronchoconstriction induced by inhalation of SO₂ in asthmatic patients (Harris et al. 1981, Sheppard et al. 1981). On the basis of these results there are reasons to doubt that SCG acts only by inhibiting mediator release. Thus, an additional mode of action must be considered (Bernstein 1981). One of the proposed mechanisms has been stimulation of the vagus via effect on "irritant receptors" (Jackson & Richards 1977b).

When SCG has been tested in guinea-pigs, many conflicting results have been obtained (for ref. see II). Most of these tests dealt with inhibition by SCG of PCA or of histamine release from chopped lung tissue. Moreover, Taylor & Roitt (1973) found that acute treatment with SCG reduced an IgE-mediated lung anaphylactic reaction in guinea-pigs challenged 12 days after sensitization. When tests were performed after prolonged sensitization periods, and when the response was due to IgG₁ and not to IgE antibodies, SCG was either inactive or even increased the anaphylactic reactivity. The present investigation shows inhibition of the "IgE"-mediated, antigen-induced bronchoconstriction after acute treatment with SCG. This effect

was seen both 14 and 40 days after sensitization. However, no effect of acute SCG treatment was seen 40 days after sensitization on an "IgG"-mediated bronchial anaphylaxis (II). An explanation of these results could be that the activation mechanism differs at the "IgE"- and "IgG"-mediated release from mast cells as indicated by the results obtained with phospholipase A₂-inhibitors (VI). Furthermore, atropine causes a partial inhibition of the "IgE"-but not the "IgG"-mediated anaphylaxis. Thus, an effect of acute treatment with SCG on "irritant" receptors could not be excluded.

In the pathogenesis of human asthma, hyperreactivity to released mediators is one of the mechanisms which has been suggested to contribute to the severity of the disease. SCG has been reported to reduce the bronchial hyperreactivity to histamine in pollen-allergic patients. This effect was claimed to develop only after several weeks of continuous prophylactic therapy (Altounyan 1980). However, there are reports which deny that mechanism of action for SCG (for ref. see Church 1978). The present investigation shows that there is an increased bronchial reactivity to methacholine but not to histamine in guinea-pigs sensitized to produce IgE-antibodies (IV). Unpublished results show that no increased sensitivity to methacholine was seen in guinea-pigs sensitized to produce only "IgG" antibodies. It has also been suggested that one reason for the hyperirritability of the airways in asthmatics might be a β -adrenoceptor blockade (for ref. see Reed & Townley 1978, Boushey et al. 1980). Decreased reactivity to isoprenaline was also observed in isolated tracheas taken from guinea-pigs sensitized to produce IgE antibodies (IV). However, the changes in reactivity to methacholine and isoprenaline were rather minor (IV).

To examine the effect of SCG on these parameters, long-term treatment was used. The results show that this type of treatment reduced the "IgE"-mediated anaphylactic bronchoconstriction and that the decreased response capacity persisted, even 3 days

BRONCHIAL ANAPHYLACTIC REACTIVITY GUINEA PIGS

EFFECT ON PERSISTENT 'ALLERGIC' STATE

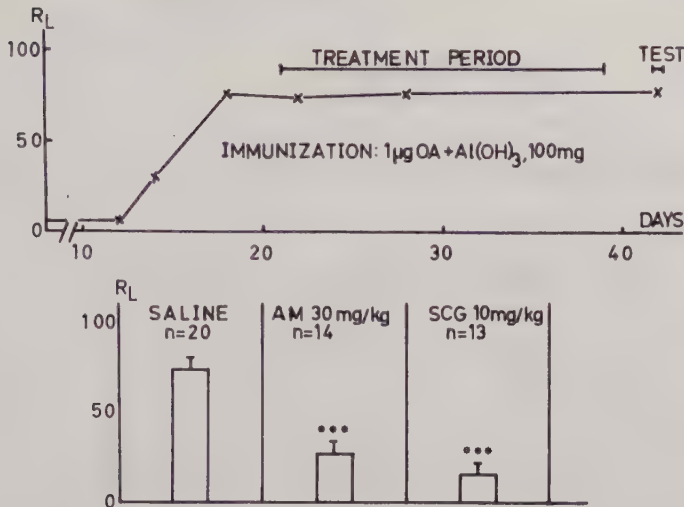


Figure 3. Effect of long-term treatment with aminophylline (AM) and sodium cromoglycate (SCG) in guinea-pigs sensitized with 1 μ g OA + Al(OH)₃, 100 mg. The animals were given AM (15 mg/kg) orally twice a day and SCG (10 mg/kg) i.p. once a day. The animals were challenged with 5 μ g/kg of OA day 42 after sensitization, 3 days after cessation of the treatment. Columns represent mean values + s.e. mean. *** 0.001>P.

after cessation of the treatment (fig. 3, IV). This effect was correlated to decreased histamine release at antigen challenge in vitro. Unpublished observations show that long-term treatment with SCG also inhibits a secondary antigen response three days after cessation of the treatment (fig. 4). The long-term treatment with SCG increased the reactivity to isoprenaline but no effect was seen on methacholine sensitivity (IV). At long-term treatment with SCG, no effect was obtained on the "IgG"-mediated bronchoconstriction after long-term treatment with SCG (IV).

Thus, long-term treatment with SCG selectively inhibits an "IgE"-mediated bronchoconstriction in sensitized guinea-pigs. An

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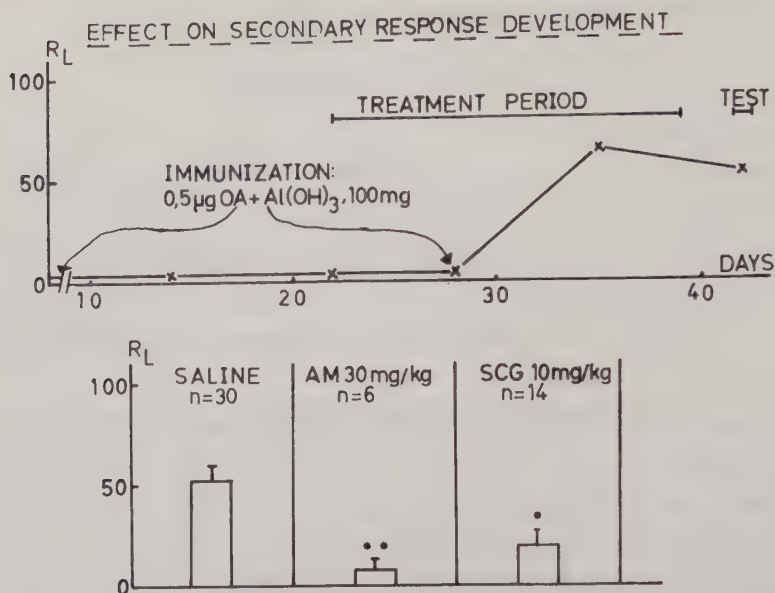


Figure 4. Effect of long-term treatment with aminophylline (AM) and sodium cromoglycate (SCG) on a booster response in guinea-pigs. The animals were primarily sensitized with 0.5 μ g OA + Al(OH)₃, 1 mg (day 0) and boosted with 0.5 μ g OA + Al(OH)₃, 1 mg (day 28). The animals were given AM (15 mg/kg) orally twice a day and SCG (10 mg/kg) i.p. once a day. The animals were challenged with 5 μ g/kg of OA day 42 after sensitization, 3 days after cessation of the treatment. Columns represent mean values + s.e.mean. N.S. = not significant. * 0.05>P>0.01, ** 0.01>P>0.001.

explanation of the decreased anaphylactic response capacity after long-term treatment with SCG could be a decrease in mediator release capacity and/or effect on "irritant" receptors. Although there is a small change in β -adrenoceptor sensitivity after long-term treatment with SCG, the restoration of changed sensitivity to mediators does not seem to be responsible for the effect. According to Cockcroft et al. (1977) no major increase in sensitivity to inhaled histamine or methacholine was seen in patients developing only immediate reactions to antigen challenge. However, those showing a dual response (immediate and late reactions) or only a late reaction developed non-specific

hyperreactivity. These results might indicate the necessity of developing an animal model showing both immediate and late reactions to estimate the role of SCG in the restoration of non-specific hyperreactivity.

2. Aminophylline (AM)

For many years, theophylline has been used in the treatment of bronchial asthma. Its beneficial effects have been ascribed to its bronchodilating properties. In the present investigation acute treatment with AM (2-32 mg/kg i.v.) inhibits a histamine-induced bronchospasm in a dose-dependent way (II). James (1967) showed that theophylline (4 mg/kg) reduced the bronchoconstriction induced by acetylcholine in anaesthetized guinea-pigs. This effect of theophylline was markedly reduced after adrenalectomy or in the presence of a β -adrenergic blocking agent indicating involvement of adrenalin. Thus, in the present investigation we cannot exclude adrenalin involvement in the AM-induced inhibition of the bronchoconstriction induced by histamine.

According to textbooks, xanthine derivatives act by increasing cAMP by inhibition of phosphodiesterase. However, during the last few years the role of theophylline as a cAMP phosphodiesterase inhibitor has been questioned (for ref. see Bergstrand 1980, Rall 1980).

In the present investigation it is shown that AM produces a clear-cut anti-anaphylactic effect in actively sensitized guinea-pigs at doses which do not affect histamine-induced bronchial contractions (II). The anti-anaphylactic effect of AM differs from that of SCG in that both "IgE"- and "IgG"-mediated reactions are inhibited (II) which indicates that the mechanism of action of the two drugs differs. The results obtained with AM might indicate mast cell release inhibition by a mechanism of

action separate from phosphodiesterase inhibition. Such an effect could be that AM interferes with receptor-mediated effects of adenosine.

Adenosine is released in hypoxic canine lung (Mentzer et al. 1975). It potentiates the release of histamine from purified rat peritoneal mast cells (Marquardt et al. 1978) or guinea-pig lung tissue (Welton & Simko 1980), but rather inhibits the release of histamine in human leucocytes (Marone et al 1979), indicating species or tissue specificity.

The increased antigen-induced histamine release from chopped guinea-pig lung tissue caused by adenosine is partially inhibited by theophylline (Welton & Simko 1980). Unpublished observations by us show that adenosine treatment in vivo causes no potentiation of the antigen-induced bronchoconstriction. This may be explained by the rapid degradation of adenosine by adenosine deaminase in the blood or tissue. 2-chloroadenosine, which binds to adenosine cell surface receptors and mimics the action of adenosine in several tissues, is metabolized at a much lower rate than adenosine is (Londos & Wolff 1977, Marquardt et al. 1978, Premont et al. 1977). Unpublished results show that this compound potentiates the antigen-induced "IgE"-mediated bronchial contraction (fig. 5). The increase in bronchial contraction is antagonized by low doses of AM (fig. 6). Therefore adenosine antagonism might be the mechanism of action of AM in these experiments. However, against this possibility speaks enprofylline, which has been shown to lack adenosine antagonistic properties in isolated guinea-pig trachea and guinea-pig myenteric plexus (Persson & Kjellin 1981), also exerts anti-anaphylactic properties in subbronchodilating doses (fig. 7, unpublished observations).

Apart from the inhibition of the anaphylactic bronchoconstriction after acute treatment of AM, the present investigation shows that AM when administered twice daily to guinea-pigs,

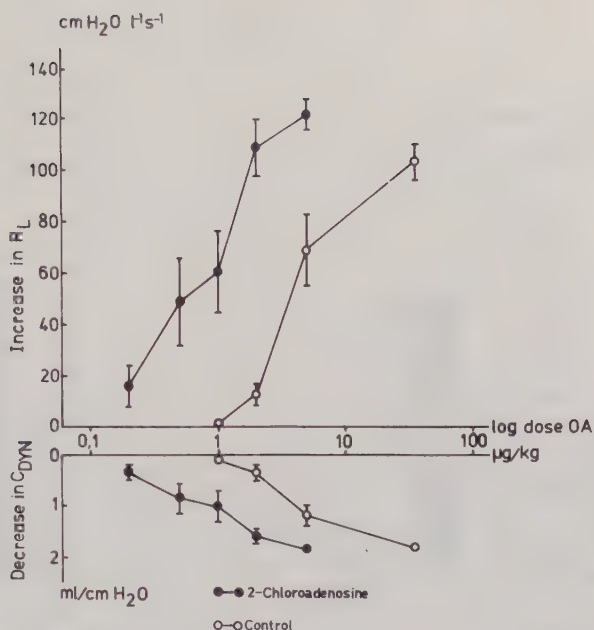


Figure 5. Antigen-induced increase in lung resistance (R_L) and decrease in compliance (C_{DYN}) after acute treatment with 2-chloroadenosine in anaesthetized guinea-pigs sensitized with $1 \mu\text{g}$ OA + $\text{Al}(\text{OH})_3$, 100 mg. The tests were performed 42 days after sensitization. $\circ - \circ$ Control. $\bullet - \bullet$ 2-chloroadenosine $30 \mu\text{g}/\text{kg}$ given i.p. 30 sec. before challenge. Potency of 2-chloroadenosine compared with saline pretreatment 0.14 (0.09 - 0.21). Figures within parenthesis indicate 95 % confidence limits. Points indicate mean values. Vertical lines indicate s.e.mean.

protects the animals from an anaphylactic bronchoconstriction even three days after the end of treatment (fig. 3, IV). At this point no serum levels of theophylline could be detected. Again AM inhibits both the "IgE"- and "IgG"-mediated anaphylactic bronchoconstriction. Unpublished observations show that long-term treatment with AM also inhibits a secondary antigen response three days after cessation of the treatment (fig. 4). As has been discussed earlier, the isolated event of increased levels of IgE antibodies appearing at atopic conditions may depend on a deficiency of suppressor T cells for the production of this immunoglobulin class. Theophylline and other inducers of cyclic nucleotides are shown to influence the number of T cells

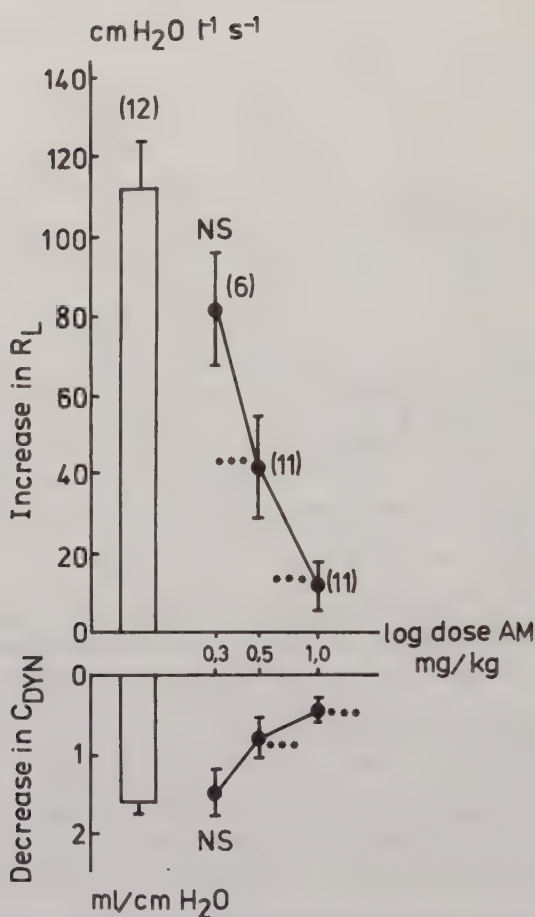


Figure 6. Effect of aminophylline (AM) on an antigen-induced bronchial anaphylaxis in guinea-pigs pretreated with 2-chloroadenosine (30 μ g/kg i.v. 30 sec. before challenge). The guinea-pigs were sensitized with 1 μ g OA + Al(OH)₃, 100 mg, and the tests were performed day 42 after sensitization. $\square$ Control response. Challenge dose 2 μ g/kg of OA given i.v. $\bullet$ - $\bullet$ dose-response for aminophylline given 2 min. before OA. Columns and points show mean values. Vertical lines indicate s.e.mean. n=8-10.
 *** 0.001 > P.

in various experimental systems. However, there are conflicting results as to whether this influence of theophylline is on the T helper or T suppressor cell population (Rola-Pleszczynski &

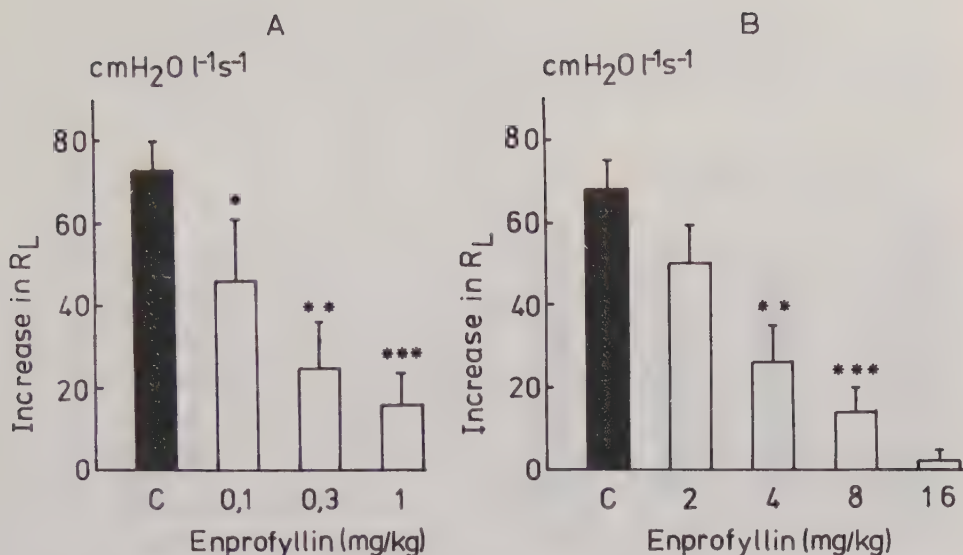


Figure 7. Anti-anaphylactic and bronchospasmolytic effects of enprofylline. A) Anti-anaphylactic effect of enprofylline. The tests were performed 14 days after sensitization of guinea-pigs with 10 μ g OA + Al(OH)₃, 100 mg. ■ Control response, 5 μ g/kg of OA given i.v. □ Dose-response for enprofylline given 2 min. before challenge. B) Bronchospasmolytic effect of enprofylline. The tests were performed in unsensitized guinea-pigs. ■ Histamine, 4 μ g/kg given i.v. □ Dose-response for enprofylline given 2 min. before histamine. Columns represent mean values. Vertical lines indicate s.e.mean. n=6.

* 0.05 > P > 0.01, ** 0.01 > P > 0.001, *** 0.001 > P.

Blanchard 1981, Shore et al. 1978, Katz & Fauci 1978, Klein et al. 1979, Gupta 1979). Perper et al. (1973) have shown that daily administration of theophylline in mice suppresses the secondary IgE antibody response. However, decreased antibody production after theophylline treatment would lead to decreased mediator release. No such effect was seen in the present investigation. The fact that normal anaphylactic reactivity was seen seven days after cessation of the treatment, and that the effect of AM was seen on both "IgG"- and "IgE"-mediated anaphylactic lung reactions, indicates that at least decreased IgE antibody

production is a less likely mechanism of action. Thus, we can offer no explanation of the present results obtained with acute or long-term treatment with AM and further experimental studies will be needed to clarify the effect. Furthermore, the clinical relevance of these effects must be established.

3. Glucocorticosteroids

Glucocorticoids can effectively relieve the symptoms of atopic diseases such as asthma and rhinitis, but the mechanisms behind this protection are still only partly understood. The well-known anti-inflammatory effect of glucocorticoids is probably important for that effect. Moreover, the recent findings that glucocorticoids inhibit the immediate reaction in man (Pare & Hogg 1980, Okuda & Mygind 1980, Martin et al. 1980, Dahl & Johansson 1981, Pipkorn 1982) and inhibit the histamine release from human basophils (Schleimer et al. 1981) and from human nasal mucosa (Pipkorn & Andersson 1982) point to an anti-anaphylactic activity.

In earlier studies on guinea-pigs sensitized to produce only IgG antibodies, there has been no evidence that glucocorticoids modify Type I allergic reactions in vivo nor was any effect on histamine release observed (Hicks 1970, Forsberg & Sörenby 1981). However, glucocorticoid pretreatment in addition to mepyramine (an H_1 -antagonist) treatment decreased the anaphylactic response in vivo more than mepyramine treatment alone did. Such inhibition has been suggested to depend on a decreased production of SRS-A as demonstrated in vitro (Goadby & Smith 1964, Forsberg & Sörenby 1981). Several groups of workers have shown that also prostaglandin release can be inhibited by glucocorticoids (Lewis & Piper 1975, Hong & Levine 1976, Flower & Blackwell 1979). Although such a mechanism can be of great value for the anti-inflammatory action of glucocorticoids, it seems to be a questionable explanation of the effect on the immediate

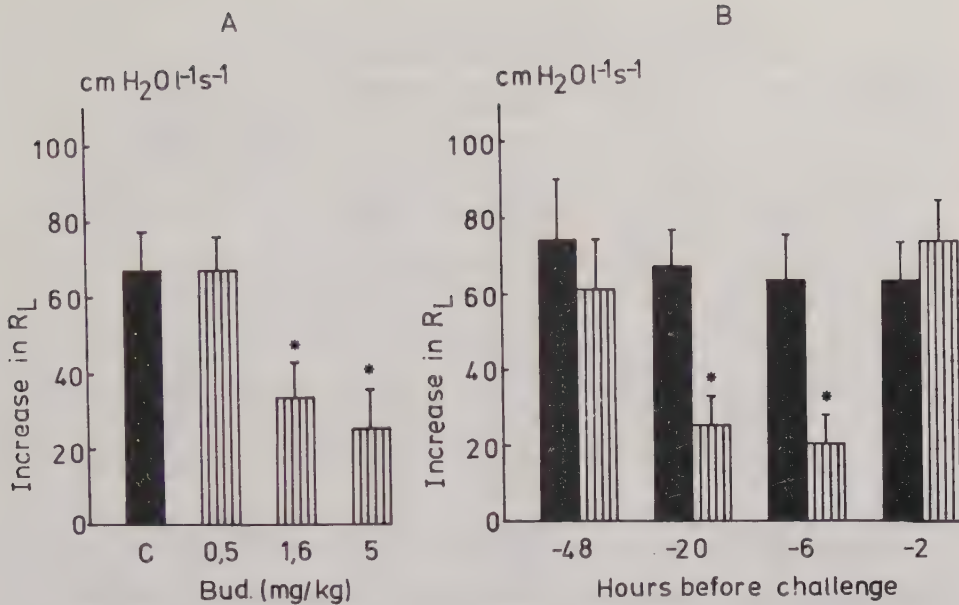


Figure 8. Inhibitory effect of budesonide on an antigen-induced bronchial anaphylaxis in guinea-pigs sensitized with 1 μ g OA + Al(OH)₃, 100 mg. The animals were challenged with 5 μ g/kg of OA i.v., day 42 after sensitization. A. Dose-response with budesonide instilled intratracheally, 20 h before challenge. ■ Control response. ▨ Budesonide pretreated.

B. Temporal development of the protective effect induced by intratracheally instilled budesonide. ■ Control response. ▨ Budesonide, 5 mg/kg.

Columns show mean results. Vertical lines indicate s.e.mean. n=6-8. * 0.05>P>0.01.

type reaction since compounds such as indomethacin are of no value in this type of reaction (for ref. see the mediator release section).

The results of the present investigation show that pretreatment with budesonide or hydrocortisone inhibited the "IgE"-mediated anaphylactic bronchoconstriction. The inhibition was seen both after i.p. injection (V) and intratracheal instillation (fig.

Table 1

EFFECT (MEAN $\pm$ S.E.M.) OF PRETREATMENT IN VIVO WITH VEHICLE OR BUDESONIDE ON HISTAMINE RELEASE FROM GUINEA-PIG LUNG.					
SENSITIZATION REGIMEN	DRUGS	PRETREATMENT PERIOD	TOTAL TISSUE HISTAMINE (μ G/G)	SPONTANEOUS RELEASE %	CHALLENGE INDUCED RELEASE. POTENCY OF BUDESONIDE COMPARED TO VEHICLE PRETREATMENT
A (1 μ G OA + AL (OH) ₃ 100 MG)	VEHICLE	15 - 20 H	8.9 $\pm$ 1.0 (N = 8)	6.4 $\pm$ 0.7 (N = 8)	0.19
	BUDESONIDE (50 MG/KG I.P.)		6.8 $\pm$ 0.7 (N = 12) N.S.	6.9 $\pm$ 0.6 (N = 12)	(0.07 - 0.51) ^A
A (1 μ G OA + AL (OH) ₃ 100 MG)	VEHICLE	5 AND 6 DAYS	8.9 $\pm$ 1.0 (N = 8)	7.3 $\pm$ 1.8 (N = 8)	0.13
	BUDESONIDE (50 MG/KG I.P.)		7.1 $\pm$ 0.6 (N = 9) N.S.	9.0 $\pm$ 1.2 (N = 9) N.S.	(0.04 - 0.38) ^A
B (5 MG OA + 10 MG OA)	VEHICLE	15-20 H	12.5 $\pm$ 0.8 (N = 5)	6.4 $\pm$ 0.8 (N = 5)	1.14
	BUDESONIDE (50 MG/KG I.P.)		13.8 $\pm$ 0.6 (N = 5) N.S.	6.3 $\pm$ 0.2 (N = 5) N.S.	(0.48 - 2.73) ^A

A = 95 % CONFIDENCE LIMITS

8A) and is probably mediated over glucocorticoid hormone receptors as it appears after a latency period of a few hours (fig. 8B). On the other hand, the glucocorticoid pretreatment gave no significant protection on the "IgG"-mediated anaphylaxis (V). The anti-anaphylactic effect of budesonide demonstrated in guinea-pigs sensitized to produce IgE antibodies correlates with the effects seen on antigen-induced histamine release in vitro (V, table 1). However, no budesonide-induced decrease of histamine release capacity could be demonstrated in chopped lung tissue from guinea-pigs sensitized to produce only IgG antibodies (table 1), which is in good accordance with the corresponding respiratory measurements.

As the anaphylactic manifestations in the guinea-pig are to a great extent caused by histamine (VI), the reduced histamine release by glucocorticoids in guinea-pigs sensitized to produce

IgE antibodies (V) is probably of very great importance for their anti-anaphylactic activity. That it is the release process that is affected is supported by the fact that no change in the total lung tissue content of histamine occurred (table 1). Such an effect has been reported by Dunskey et al. (1979) and Shah et al. (1980). It is also shown that SRS-A participates in the antigen-induced, "IgE"-mediated bronchoconstriction (VI). Furthermore, the inhibitory effect obtained with budesonide on the "modified" -"IgE"- mediated bronhoconstriction (VI), in the presence of an H₁-antagonist to decrease the importance of histamine, shows that reduced histamine release is not the only reason for the anti-anaphylactic activity of glucocorticoids. As that spasm is blocked by FPL 55712 an effect on SRS-A release is suggested.

One of the proposed mechanisms of action for glucocorticoids is inhibition of the enzyme phospholipase A₂ by formation of a specific protein, macrocortin (Blackwell et al. 1978). The role of phospholipase A₂ in the release of histamine and products of arachidonic acid metabolism has been discussed earlier in this thesis (for ref. see the mediator release section). In the present investigation it is shown that histamine and SRS-A participate in both the "IgE"-and the "IgG"-mediated bronchospasm in the guinea-pig. However, it is also shown that the proposed phospholipase A₂ inhibitors mepacrine and p-bromphenacylbromid inhibited only the "IgE"- but not the "IgG"-mediated bronchospasm. In accordance with the results and suggestions above, budesonide did not protect against bronchoconstriction induced by arachidonic acid infusion. It is possible that budesonide and hydrocortisone inhibit the anaphylaxis by interacting with early events in the triggering of the IgE receptor: it has recently been shown that there are different Fc receptors for IgE and IgG antibodies (Daeron et al. 1980) and protection against anaphylaxis cannot be mediated on the IgG receptor (V). Such a hypothesis is in accordance with recent findings. Phospholipase A₂, besides its function to induce histamine release

and to liberate arachidonic acid from membrane-bound phospholipids, has also been proposed to play a part in the regulation of the so called IgE-binding factor, which is involved in the IgE-induced expression of Fc ϵ receptors and regulates the IgE response in rat T lymphocytes (Yodoi et al. 1981a). Interestingly, glucocorticoids also prevent the IgE-induced expression of IgE receptors and change the nature of the IgE-binding factors formed (Yodoi et al. 1981b). Glucocorticoid treatment has also been reported to reduce the number of monocytes with Fc receptors for IgE in patients with severe atopic disease (Melevicz et al. 1981). Whether this is due to a decreased number of IgE receptors per cell or to a changed cell traffic as reported by Parrillo & Fauci (1979) is not established. However, Crabtree et al. (1979) and Suzuki et al. (1980) report that glucocorticoids decrease the number of Fc γ receptors on granulocytes and β -lymphocytes. The time lag for these effects agrees with what is considered necessary for glucocorticoids to exert their effect. The results in the present investigation, showing that no effect is seen on an "IgG"-mediated anaphylaxis, might perhaps rule out the hypothesis of decreased Fc receptors as a mechanism for glucocorticoid action.

To further evaluate the proposed specific background for the glucocorticoid-induced protection, complementary investigations were performed. There are reports indicating that the anti-asthmatic activity of glucocorticoids may be related to their anti-spasmodic activity (Geddes & Lefcoe 1973) or to their interactions with catecholamines (Pun et al. 1973). However, we found no protective activity of budesonide on histamine- or methacholine-provoked contractions (fig. 9). Thus, budesonide does not protect by inhibiting the bronchoconstriction at the smooth muscle level. Nor did budesonide potentiate in vivo bronchorelaxation mediated by β -adrenoceptors (fig. 10). The enhancing effect of hydrocortisone on the bronchospasmolytic action in vitro of isoprenaline (table 2) does not fulfil the characteristics of an action mediated over glucocorticoid

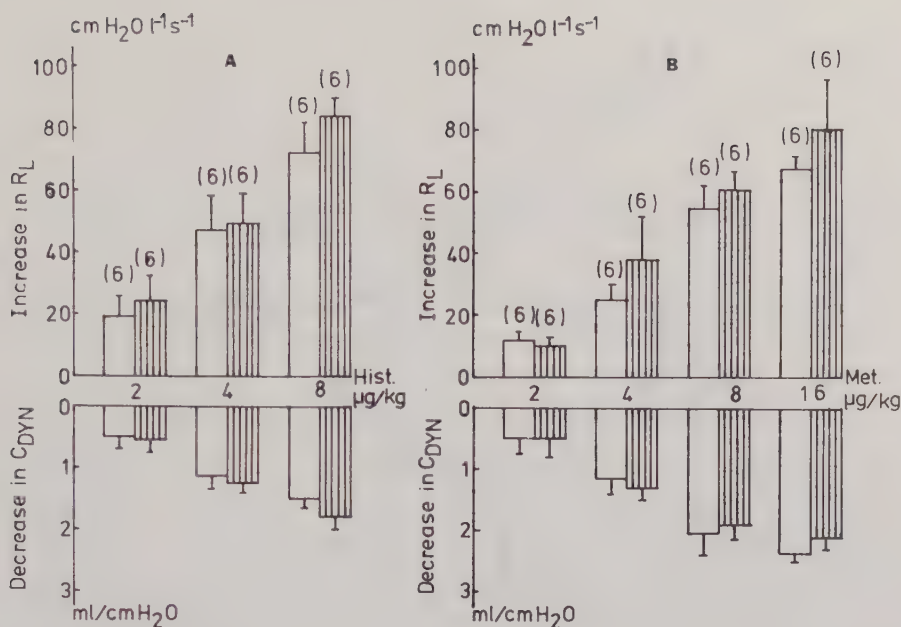


Figure 9. Effect of budesonide pretreatment (50 mg/kg, given i.p. 20 h before test) on histamine (A) and methacholine (B) sensitivity in guinea-pigs sensitized by 1 μg OA + Al(OH)₃, 100 mg. □ Control response for histamine and methacholine given i.v. ▨ Effect of histamine and methacholine in budesonide pretreated guinea-pigs. Columns represent mean results. Vertical lines indicate s.e. mean. Figures within parenthesis indicate number of experiments.

receptors (Harding 1980) as the potentiation occurs within minutes. According to previous investigations such a potentiation has not been obtained with glucocorticoids of higher potency (Svedmyr 1980).

One of the proposed mechanisms behind the effect of glucocorticoids on atopic conditions, has been decreased antibody production (Baxter & Forsham 1972). However, we noted no effect of budesonide pretreatment on the levels of circulating antibodies (V). Also the time course of glucocorticoid effect seen after instillation probably rules out that mechanism.

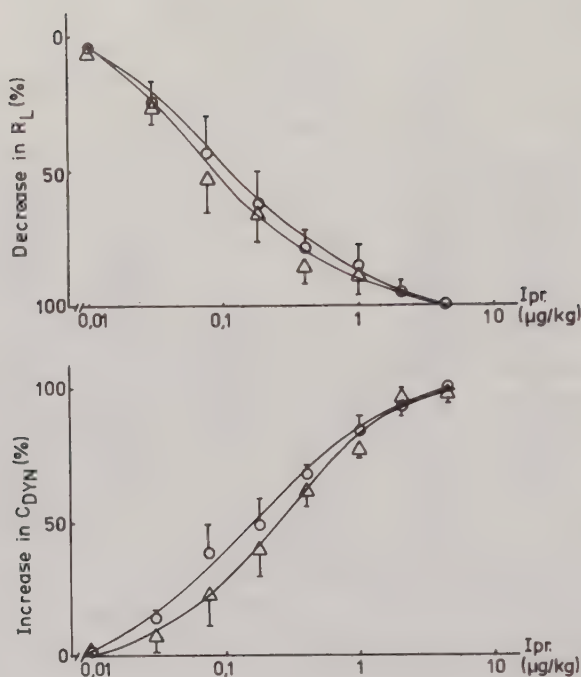


Figure 10. Effect of isoprenaline in guinea-pigs sensitized with 1 μg OA + $\text{Al}(\text{OH})_3$, 100 mg and pretreated with budesonide (50 mg/kg i.p. 20 h before test). Contraction was produced by continuous i.v. infusion of serotonin (10–15 $\mu\text{g/kg/min.}$). ○-○ Control response, untreated guinea-pigs. △-△ Budesonide pretreated guinea-pigs. Points represent mean results. Vertical lines indicate s.e.mean. $n=4$.

Thus, the results obtained in the present investigation indicate that the anti-anaphylactic activity of glucocorticoids in guinea-pigs sensitized to produce IgE antibodies, mainly occurs by a reduction of mediator release. To determine the exact mechanism behind the inhibition further studies are needed. The protective effects against the "IgE"-mediated anaphylaxis in guinea-pigs is in good accordance with the inhibiting effect of budesonide (given as aerosol 1.0 mg/day for 7 days; Dahl & Johansson 1981), and prednisolone (40 mg given as tablets for 7

Table 2

ACUTE EFFECTS OF BUDESONIDE ON ISOPRENALINE-INDUCED RELAXATION OF TRACHEAS
IN VITRO. THE TRACHEAS WERE CONTRACTED BY 1×10^{-6} MOL/L CARBACHOLINE.

TREATMENT	CORTICOID MOL/L	N	ED ₅₀ FOR ISOPRENALINE-INDUCED RELAXATION, NMOL/L		POTENCY OF TEST TREATMENT
			VEHICLE	TEST	
VEHICLE/ VEHICLE	-	4	137 (118 - 157)	140 (111-173)	0.98 (0.78 - 1.23)
VEHICLE/ BUDESONIDE	6.5×10^{-5}	4	120 (103-138)	96 (83-114)	1.27 (1.04 - 1.52)

() = 95 % CONFIDENCE LIMITS

days; Martin et al. 1980) on the immediate bronchial reaction in challenged allergic patients. The lowest effective dose of budesonide in the guinea-pig is 1.6 mg/kg given as intratracheal instillation, which reduces the anaphylactic reaction by 50 % $P < 0.01$ (fig. 8A). As it is not yet known if intratracheal instillation represents mainly systemic or local administration to the lung tissue, we still cannot compare the necessary doses in the guinea-pig model and in the patients. We think that an adequate inhalation technique for glucocorticoids is needed to further evaluate the relevance of the glucocorticoid effects for the immediate bronchial reactions in clinical asthma.

In conclusion, the results in the present investigation show that well-known anti-asthmatic compounds like SCG, aminophylline and glucocorticoids inhibit the antigen-induced "IgE"-mediated immediate type bronchoconstriction. The effects seen correlate

well with the clinical effects obtained with these compounds. Thus, this model is considered to be a good one for evaluation of drug effects on the acute anaphylactic reaction. The mechanism behind the anti-asthmatic effect of these compounds is not completely understood. Although no precise mechanism of action is shown in the present investigation we think that the antigen-induced, "IgE"-mediated bronchoconstriction is a promising model for further studies. It will be of particular interest to study the role of SCG in inhibiting "irritant receptors". In the literature, the importance of phospholipase A₂ for the induction of the IgE-mediated release process is stated. According to the present investigation, the proposed phospholipase A₂ inhibitors mepacrine, p-bromphenacylbromide, and the glucocorticoids budesonide and hydrocortisone inhibit the "IgE"- but not the "IgG"-mediated anaphylaxis. The same pattern of response is obtained with SCG. Interestingly, many years ago SCG was shown to inhibit a phospholipase A₂-induced histamine release (Orr & Cox 1969). Thus, the importance of inhibiting early events in the IgE-mediated release process, like phospholipase or methyltransferase inhibition, is indicated. Furthermore, the modified "IgE"-induced bronchospasm, which is shown to be almost completely mediated by SRS-A, is considered to be a valuable model for evaluation of SRS-A antagonists.

SUMMARY

This thesis characterizes a model of reagin-mediated bronchial anaphylaxis in guinea-pigs designed to be used in research on anti-asthmatic drugs.

Bronchial anaphylactic responses were estimated as change in pulmonary resistance and dynamic lung compliance in anaesthetized animals at intravenous challenge with the specific antigen ovalbumin.

A state of anaphylactic response capacity was induced by i.p. injections of OA together with alum. 1 μ g of OA was found to be an optimal dose for induction of a persistent state of responsiveness; lower doses of OA did not induce responsiveness, whereas higher ones induced responsiveness which faded within three to four weeks. In animals primed with low doses of antigen, booster injections led to the development of anaphylactic responsiveness. Pretreatment of animals with cyclophosphamide two days before a primary or a secondary antigen injection prolonged the ensuing anaphylactic response capacity. Analyses of serum homocytotropic antibody titre by PCA tests indicated that IgE-like antibodies mediated anaphylactic responsiveness after this kind of immunization and that pretreatment with cyclophosphamide increased IgE biosynthesis.

Immunization of guinea-pigs with high doses of OA without alum, induced anaphylactic response capacity which was found to be mediated by IgG₁-like antibodies. An approximately ten times higher dose of OA was needed to induce bronchoconstriction in this system than in the IgE-mediated one.

The use of mediator antagonists indicated that both types of anaphylaxis are due to antigen-induced release of histamine and SRS-A from mast cells. However, participation of prostaglandins and thromboxanes seems to be small.

Various drugs affected anaphylactic responses in the two systems in different ways. Proposed phospholipase A₂ inhibitors inhibited the "IgE"- but not the "IgG"-mediated lung anaphylaxis. This suggests different activation mechanisms for the anaphylactic lung reaction mediated by IgE and IgG antibodies, resp. Acute treatment with SCG and the glucocorticoids budesonide and hydrocortisone blocked the IgE-mediated, but not the IgG-mediated, anaphylactic reaction, whereas AM in doses that did not inhibit a histamine-induced bronchoconstriction, blocked both. The effect of budesonide was observed when drug administration was performed 6 or 20 hours before antigen challenge; lung tissue from budesonide-treated animals released less histamine than controls at antigen challenge in vitro.

Animals of the "IgE"- but not those of the "IgG"-type which had been treated for three weeks with SCG responded less well to antigen challenge than saline treated controls did. This difference remained up to seven days after the completion of SCG treatment. In concordance with this, lung tissue from SCG treated "IgE"-type animals showed impaired histamine release capacity. Animals treated with AM showed reduced response capacity to antigen three days after the completion of treatment; this effect was observed in "IgE"-type as well as "IgG"-type animals. The capacity to release histamine from lung tissue in vitro was not impaired in these animals.

It is concluded that the bronchial anaphylaxis in guinea-pigs sensitized with a low dose of OA (1 µg) together with alum, 6-7 weeks before the experiments, is a good model for evaluation of drug effects on the acute anaphylactic reaction.

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